NANOBACTERIA IN CARBONATES. Carlton C. Allen[1], Kathie L. Thomas-Keprta[1], David S. McKay[2], and Henry S. Chafetz[3] [1]Lockheed Martin Engineering & Sciences, Houston, TX 77058 [2]NASA Johnson Space Center, Houston, TX 77058 [3]University of Houston, Houston, TX 77204

Thermal spring deposits have often been cited as prime locations for exobiological exploration [1]. Specialized bacteria thrive in the heated, mineralized waters and precipitates of carbonate and silica from these waters often trap and preserve the bacteria [2]. Possible relic biogenic forms have recently been discovered in carbonate deposits within martian meteorite ALH84001 [3]. Unique carbonates have also been described in the white druse of martian meteorite EETA79001 [4]. We are studying possible bacteria preserved in thermal spring carbonate deposits as a modern analog to such preservation below the surface of Mars.

Carbonates. We are studying travertine samples from Le Zitelle spring in central Italy. Le Zitelle is an active thermal spring with calcium carbonate precipitation rates greater than 2 mm per day [5]. Three travertine samples (ZA, ZE, ZF) were collected from sites with water temperatures of 59.0, 43.6, and 35.3°C, respectively [6]. Chips of travertine were coated with Au-Pd for high resolution SEM. Selected samples were etched for 60 s in 1% HCl, rinsed in triply distilled water, and air dried prior to coating. Figures 1-3 show etched surfaces.

The travertine samples are intimate intergrowths of two calcium carbonate minerals, calcite and aragonite [5]. The calcite occurs as elongated, prismatic crystals tens of micrometers in length. The aragonite takes the form of rosettes with spherical centers and delicate radiating needles 10-20 μm long (Figure 1). The precipitation of aragonite versus calcite is favored by high saturation states, water warmer than 40-45°C and Mg/Ca molar ratios greater than 1 [5].

Nanobacteria. Folk [7] studied travertines from Le Zitelle spring with the SEM and discovered clusters of spheroidal objects which he called "nanobacteria." These objects ranged from 100-500 nm in diameter, significantly smaller than the size range of 500-3000 nm traditionally cited for modern bacteria [8].

Our samples contain two distinct types of spheroids equivalent to Folk's nanobacteria. Figure 2 shows several clusters of the larger type, which typically range from 200-400 nm in diameter. These occur as individuals, short chains, or small clusters on the surfaces of etched calcite crystals. The second type (Figure 3) consists of dense clusters of spheroids 50-100 nm in diameter. These fill the centers of aragonite rosettes, and are only apparent when some of the overlying aragonite is removed by etching.

Biofilm. Many crystals in the travertine samples are coated with a thin layer of "mucus" [7]. This material matches the description of microbial biofilm, an extracellular polymer secretion produced by many types of bacteria. Biofilms are commonly found in etched carbonate precipitates [9]. These films provide unique microniches in which populations of bacteria exist, protected from otherwise hostile environments [10].

All of our samples contain abundant electron-translucent biofilm (Figure 4). This material forms thin fibers as well as sheets tens of micrometers across. It coats both calcite and aragonite crystals. Biofilm is resistant to attack by 1% HCl. Most biofilms are free of spheroidal objects, though some contain an abundance of the smaller spheres.

Life Forms? No investigator has yet demonstrated whether the spheroids in Le Zitelle travertine are living bacteria, fossils, spores, or abiotic precipitates. The spheroids are significantly smaller than the lower limit generally accepted for bacteria. They are also too small for elemental analysis by SEM. Efforts to culture bacteria from these samples have met with only limited success [5].

Our ongoing research includes developing techniques to isolate individual spheroids from the underlying carbonates. This will allow high resolution TEM imaging combined with crystallographic and elemental analysis. We are attempting to culture bacteria from the travertines using conditions and media specific to thermal spring samples [11]. We are also initiating a program to search for DNA in the travertine spheroids, to isolate and sequence this DNA, and to compare it to that of known bacteria.

Implications. Thermal spring deposits on Mars are prime sites in which to search for evidence of microbial life. Active springs such as Le Zitelle provide "best case" terrestrial analogs for understanding such environments. Their carbonate deposits appear to contain abundant nano-scale microbes. Proof that these are indeed biological will extend the accepted size limits for life on Earth.

The thermal spring spheroids have dimensions similar to the possible relic life forms in ALH84001 [3]. Layers resembling biofilms have recently been reported in this meteorite [12]. Both types of structures were apparently formed and preserved in carbonates. The smaller Le Zitelle spheroids are closely associated with fibrous, radiating aragonite. Calcium carbonate minerals of similar morphology and size have been reported in the white druse of EETA79001 [4]. These minerals warrant further study as possible indicators of biogenic alteration on Earth or past life on Mars.

References. [1] An Exobiological Strategy for Mars Exploration (1995) *NASA SP-530*. [2] Walter, M. R. and Des Maris, D. J. (1993) *Icarus* **101**, 129-143. [3] McKay, D. S. et al. (1996) *Science* **273**, 924-930. [4] Gooding, J. L. and Wentworth, S. J. (1991) *Lunar Planet. Sci. XXII*, 461-462. [5] Folk, R. L. (1994) *Geograph. Phys. et Quat.* **48**, 233-246. [6] Chafetz, H. S. and Lawrence, J. R. (1994) *Geograph. Phys. et Quat.* **48**, 257-273. 462. [7] Folk, R. L. (1993) *J. Sed. Pet*. **63**, 990-999. [8] Hoffman, H. J. and Schopf, J. W. (1983) in Schopf, J. W. ed., *Earth's Earliest Biosphere*, Princeton Univ. Press, p. 321-360. [9] Defarge, C. et al. (1996) *J. Sed. Res*. **66**, 935-947. [10] Costerton, J. W. et al. (1994) *J. Bacteriology* **176**, 2137-2142. [11] Combie, J. and Runnion, K. (1996) *J. Indust. Microbiol.* **17**, 214-218. [11] Steele, A. et al. (1997) *Lunar Planet. Sci. XXVIII*, this volume.

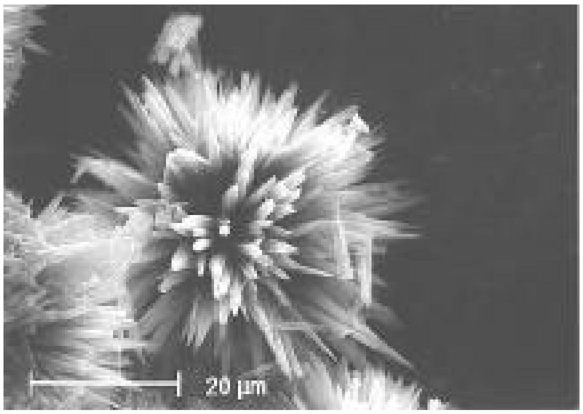

Figure 1. Aragonite rosette

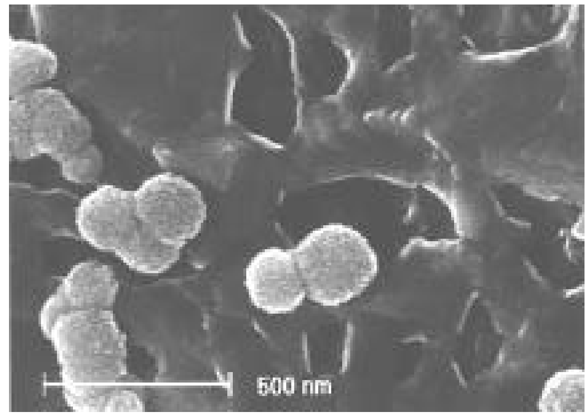

Figure 2. Large spheroids on calcite

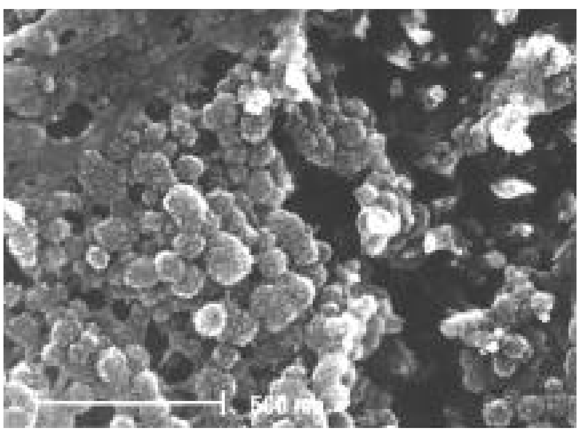

Figure 3. Small spheroids in aragonite

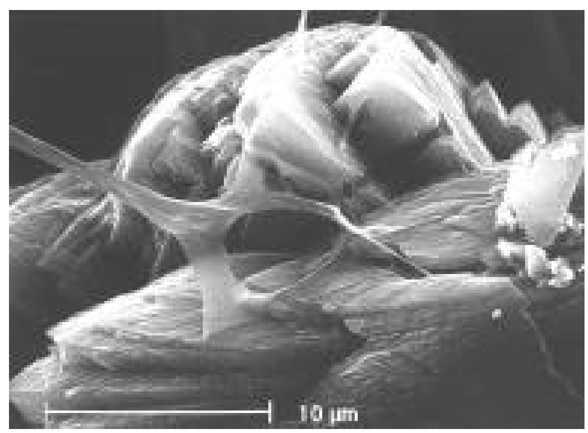

Figure 4. Biofilm on calcite